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Time-dependent changes in inner medullary plasma flow rate during potassium-depletion

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Time-dependent changes in inner medullary plasma flow during potassium-depletion. Renal concentrating ability becomes impaired after approximately 2 weeks of dietary potassium (K) depletion in the rat. Since inner medullary plasma flow (IMPF) has been shown to be reduced after 3 weeks of K-depletion, IMPF was measured after 2 and 3 weeks of dietary K-deprivation to determine if the change in IMPF is present at the time the renal concentrating defect first appears. In the present study, similar reductions in maximal urine concentration were present in rats K-depleted for 2 and 3 weeks. IMPF measured by the ^{125}I albumin accumulation method, however, was normal after 2 weeks of K-depletion (control, 35.1 ± 1.93 vs. K-depletion 2 weeks, 32.8 ± 1.52 ml/min/100 g IM), and was reduced after 3 weeks of this dietary regime (K-depletion, 3 weeks: 13.8 ± 1.84). To determine the mechanism of the decrease in IMPF after 3 weeks of K-depletion, rats were treated acutely with indomethacin. There was no significant change in IMPF in control or 3-week K-depleted rats following treatment with indomethacin. These results suggest that the reduction in medullary solute content after 2 weeks of K-depletion cannot be attributed to a reduction in IMPF. In addition, products of the cyclooxygenase enzyme systems do not appear to contribute in a major way to the reduction in IMPF measured after 3 weeks of dietary K-depletion.

Modifications dépendantes du temps du débit plasmatique médullaire interne lors de la déplétion en potassium. Le pouvoir de concentration du rein s'altère après environ 2 semaines de déplétion alimentaire en potassium (K) chez le rat. Puisqu'il a été montré que le débit plasmatique médullaire interne (IMPF) est réduit après 3 semaines de déplétion en K, IMPF a été mesuré après 2 et 3 semaines de restriction alimentaire en K afin de déterminer si la modification d'IMPF existe au moment ou le trouble de concentration rénale apparaît pour la première fois. Dans cette étude, des réductions identiques de la concentration urinaire maximale étaient présentes chez des rats déplétés en K depuis 2 et 3 semaines. Cependant, IMPF mesuré par la méthode de l'accumulation de ^{125}I-albumine était normal après 2 semaines de déplétion en K (contrôle, 35,1 ± 1,93 contre déplétion en K 2 semaines, 32,8 ± 1,52 ml/min/100 g IM) et était réduit après 3 semaines de ce régime alimentaire (déplétion en K, 3 semaines: 13,8 ± 1,84). Afin de déterminer le mécanisme de la diminution d'IMPF après 3 semaines de déplétion en K, des rats ont été traités en aigu avec de l'indométhacine. Il n'y avait pas de modification significative d'IMPF chez les rats contrôles, ou déplétés en K pendant 3 semaines après traitement par l'indométhacine. Ces résultats suggèrent que la réduction du contenu médullaire en solutés après 2 semaines de déplétion en K ne peut être attribuée à une réduction d'IMPF. En outre, les produits des systèmes enzymatiques de la cyclooxygénase ne paraissent pas contribuer de façon importante à la réduction d'IMPF mesurée après 3 semaines de déplétion alimentaire en K.

An impairment of urine concentrating ability associated with hypokalemia in humans [1] and animals [2, 3] has been known for over 20 years. Since the production of a maximally concentrated urine depends on extrarenal as well as renal mechanisms, the defect caused by potassium (K) depletion has been approached from several viewpoints. The results of some of these investigations have been reviewed elsewhere [4]. Despite the continuing interest in elucidating the mechanism of the concentrating defect due to K-depletion, a significant degree of controversy exists in major research areas. For example, although there is evidence from in vitro experiments that the generation of cyclic AMP in response to ADH is impaired in hypokalemic animals [5, 6], results from in vivo experiments fail to show that inner medullary collecting duct response to ADH is impaired in hypokalemic hamsters [7] and rats [8]. If collecting duct response to ADH is not impaired in K-depletion, then failure to maintain adequate medullary solute could be the cause of reduced urine osmolality. The reduction in medullary solute content in K-depletion [3] could be due to failure to transport adequate amounts of urea and sodium chloride into the interstitium or dissipation of the gradient due to a change in inner medullary plasma flow (IMPF) [9–11]. The finding that IMPF was reduced in rats K-depleted for 21 days [12] was thought to reflect a lower delivery of plasma to juxtamedullary nephrons which may reduce solute delivery to the loop of Henle. In this manner, the reduction in medullary solute content could be explained, in whole or in part, by decreased perfusion of juxtamedullary nephrons.

Dietary potassium deprivation produces a spectrum of time-dependent changes in renal and systemic function. For example, decreases in plasma K concentration and aldosterone secretion, and increases in distal nephron K reabsorption, occur within the first 3 days of K restriction [13], while polydypsia and the renal concentrating defect occur approximately on days 7 and 14, respectively [14]. If the decrease in IMPF in K-depleted rats is associated with the initiating factor responsible for the development of the concentrating defect, then this change in renal hemodynamics should be present after only 2 weeks of K-depletion at which time the defect first appears. It is the purpose of the present investigation to determine if a change in IMPF is associated with the appearance of the renal concentrating defect in rats K-depleted for only 14 days.

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Regarding the mechanism of the decrease in IMPF in rats K-depleted for 3 to 4 weeks duration, there is evidence that products of the cyclo-oxygenase enzyme system may affect IMPF [9]. In addition, inner medullary production of the vasoconstrictor, thromboxane A_2 has been shown to be increased in tissue derived from K-depleted rats [15]. Therefore, it is also the purpose of this study to examine the effect of inhibition of the cyclo-oxygenase enzyme system on IMPF in rats K-depleted for 3 to 4 weeks.

The results of the present study show: *(1)* IMPF is normal after 2 weeks of K-depletion at a time when the renal concentrating defect is well established. *(2)* As the duration of K-depletion continues, the renal concentrating defect does not worsen, however, IMPF decreases confirming the previous report by Whinnery and Kunau [12]. *(3)* Treatment of rats K-depleted for 3 to 4 weeks with indomethacin does not significantly increase IMPF.

Methods

Experiments were performed on 69 male Munich-Wistar rats (Timco Breeding Laboratories, Houston, Texas) weighing 170 ± 4.2 g. Animals were fed a K-deficient diet (ICN Nutritional Biochemical Division, International Chemical and Nuclear Corp., Cleveland, Ohio) or a diet of normal K content [potassium, 4 mM/kg diet, added to the K-deficient diet (ICN)]; both were supplemented with magnesium. It is well established that water intake increases and food intake decreases in animals consuming a K-deficient diet. In a preliminary balance study, food and water intakes were measured in rats consuming either the control or K-deficient diet. Rats consumed approximately 6 g of K-deficient diet per 100 g of body weight per day. Control rats drank approximately 20 ml/100 g body weight per day. Therefore, all rats were given and consumed only 6 g of diet per 100 g body weight per day; water intake in the rats consuming the K-deficient diet was restricted to 20 ml/100 g body weight per day. Animals were subsequently divided into five experimental groups: *(1)* Control rats (N = 17) consumed a measured quantity of normal K diet as described above and drank tap water. *(2)* Rats K-depleted 16 days (N = 19) consumed a measured quantity of K-deficient diet and were given and consumed 20 ml/100 g body weight per day of distilled water for 14 to 17 days (m = 15.7 ± 0.21). *(3)* Rats K-depleted 24 days (N = 15) were treated the same as group 2 but were maintained on this dietary regimen for 22 to 30 days (m = 24.3 ± 1.09). *(4)* Control rats given indomethacin (N = 9) were treated the same as group 1 but received indomethacin on the day of the experiment. *(5)* Rats K-depleted 24 days (N = 9) were treated the same as group 3, but received indomethacin on the day of the experiment.

Maximum urine concentrating ability. After a minimum of 14 days on the control diet, and after 14 and 21 days of K depletion, maximum urine concentration was measured in the first urine sample collected following 15 hr of dehydration. Animals were returned to their cages and allowed 24 to 48 hr before any further experiments were performed.

Clearance experiments. GFR, C_{PAH}, urine flow rate, and K-excretion were measured in (6 to 11 animals per group) in control and K-depleted rats (groups 1 to 5). Animals were anesthetized by intraperitoneal injection of ketamine hydrochloride (Parke Davis and Co., Detroit, Michigan) 50 mg/kg and Inactin 20 mg/kg (Byk-Gulden, Konstanz, West Germany) and placed on a thermostatically controlled table. A tracheostomy was performed. A PE-10 catheter for the infusion of additional anesthetic when needed and a PE-50 catheter were inserted into the same jugular vein. The PE-50 catheter was used for the infusion of inulin and ^{3}H PAH containing solutions during the clearance measurements and the subsequent infusion of ^{125}I-albumin for the measurement of IMPF. Another PE-10 catheter for the infusion of FD+C green dye number 3 (50 mg/ml, Keystone Aniline and Chemical Co., Chicago, Illinois) for the purpose of measuring renal transit time was inserted into the other jugular vein. A suprapubic incision was made, the bladder was externalized, and a PE-60 catheter with a flared end was inserted. The left femoral artery was catheterized with a length of PE-50 tubing for blood collection and continuous monitoring of blood pressure (BP). Only data from animals with mean arterial BP greater than 90 mm Hg throughout the experiment were accepted.

As soon as the jugular catheters were inserted, the animal was given 1 ml of 0.9% NaCl containing 3 μCi of ^{3}H PAH and 10 mg of inulin as a priming dose and an infusion of the same solution began at the rate of 1.5% body weight per hour. Plasma levels of ^{3}H PAH were maintained approximately 100 × background. Plasma levels of inulin were maintained between 20 to 50 mg/dl. Following a 30-min equilibration period, urine samples were collected into a weighed tube for 30 min. A blood sample was taken at the beginning and the end of the urine collection.

Inner medullary plasma flow. Studies were performed in control and experimental animals immediately following the inulin and PAH clearance experiments. In these animals inner medullary plasma flow was measured by the ^{125}I-albumin accumulation method initially described by Lilienfield, Masazini, and Bauer [16] and later modified by Solez et al [17]. Following isolation of the kidneys and placement of silk ligatures (3-0) about the renal pedicle, a microscope was positioned over the kidney to assist in determining the time of arrival of ^{125}I-albumin. Before the infusion of ^{125}I-albumin was initiated, the transit time of FD+C green was taken. If proximal transit time was greater than 15 sec, the appearance of distal tubules was greater than 45 sec or dye was retained in the tubules for more than 2 min, the experiment was terminated. The PE-50 catheter in the jugular vein was then attached to a syringe containing 0.9% NaCl with 10 μCi/ml of ^{125}I-albumin (Mallincrodt, St. Louis, Missouri) colored with 25 mg/ml of FD+C green dye on the infusion side of the reciprocal action pump (Harvard Apparatus, Millis, Massachusetts). The femoral arterial catheter was attached to the withdrawal side of the pump. After the pump was started, timing of the perfusion began when the dye reached the kidney. All perfusions were performed for 20 sec. Simultaneously, the pump was stopped, and the renal ligatures were pulled tight. The kidneys were weighed after excess blood was expressed and then using a microscope, the entire inner medulla was removed as one piece. The inner medulla was weighed and then counted along with 25 μl of arterial plasma collected during the perfusion period.

Treatment with indomethacin. To determine if the decrease in inner medullary plasma flow measured in rats K-depleted for 24 days was due to increased levels of vasoconstrictor products of the cyclo-oxygenase enzyme system, control and long-term

Table 1. The effect of the duration of dietary K-depletion on body weight, plasma [K], urinary K excretion, urine flow rate, GFR, C_{PAH}, and the extraction ratio of PAH

Treatment	BW *g*	$[K]_P$ *mM*	$U_K \cdot V$ *μM/min/g KW*	V *μl/min/g KW*	GFR *ml/min/g KW*	GFR *ml/min/100 g BW*	C_{PAH} *ml/min/g KW*	Ext PAH[a]
Group 1	190	4.5	0.482	3.9	1.04	0.87	2.71	0.78
Control	±6.5	±0.042	±0.1089	±0.06	±0.084	±0.090	±0.187	±0.013
	(10)	(10)	(10)	(10)	(8)	(8)	(10)	(7)
Group 2	192	2.3[b]	0.015[b]	3.6	0.53[c,e]	0.60	1.21[b]	0.81
K-Depleted	±13.6	±0.22	±0.0030	±0.060	±0.049	±0.064	±0.122	±0.019
16 days	(13)	(11)	(11)	(11)	(11)	(11)	(11)	(6)
Group 3	147	1.7[b]	0.018[b]	5.2	0.52[d]	0.68	1.25[b]	0.75
K-Depleted	±13.8	±0.054	±0.0094	±0.10	±0.091	±0.136	±0.197	±0.019
24 days	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(9)
Group 4	180	4.0	0.465	4.2	1.02	0.82	3.00	0.73
Control	±8.4	±0.156	±0.1388	±0.04	±0.174	±0.058	±0.341	±0.044
+ Indo	(7)	(6)	(6)	(6)	(4)	(4)	(6)	(5)
Group 5	171	2.1[b]	0.011[b]	2.8	0.59[c,e]	0.69	1.35[b]	0.61[f]
K-Depleted	±3.5	±0.18	±0.0028	±0.04	±0.174	±0.197	±0.178	±0.017
+ Indo	(9)	(8)	(7)	(7)	(8)	(8)	(7)	(4)
	NS			NS		NS		

Abbreviations and symbol: KW, kidney weight; BW, body weight; Indo, indomethacin; ( ), number of animals.
[a] The values refer to the extraction ratio of PAH.
[b] $P < 0.01$ compared to groups 1 and 4.
[c] $P < 0.01$ compared to group 1.
[d] $P < 0.05$ compared to group 1.
[e] $P < 0.05$ compared to group 4.
[f] $P < 0.01$ compared to all groups.

K-depleted rats (groups 4 and 5) were given an intraperitoneal dose of indomethacin, 5 mg/kg. The rats were anesthetized as described previously; indomethacin was added to the perfusion to deliver 5 mg/kg/hr, and the clearances of inulin and PAH were measured. Inner medullary plasma flow was measured as described at least 1.5 to 2 hr after treatment with indomethacin was initiated.

PAH extraction ratio and inner medullary tissue water. In separate experiments, PAH extraction was measured in four to nine animals per group from groups 1 to 5. Rats were anesthetized with Inactin and ketamine and catheters were inserted as described previously. Thirty minutes after the initiation of the intravenous infusion, the abdomen was opened by a midline incision, and the left renal vein was exposed. Great care was taken to maintain intra-abdominal temperature at 37°C. Tubular transit time was measured and the experiment was terminated if it was not within the range of normal. A sample of 0.1 ml of renal venous blood was drawn by puncture of the vein with a 30-gauge needle, which was followed immediately by a collection of 0.1 ml of femoral arterial blood. Two to three such paired collections of renal venous and arterial blood were made in these studies. When blood sampling was completed, ligatures which had been placed around the renal pedicles were pulled tight, and the kidneys were removed and weighed as described. The inner medulla was removed from each kidney and placed in a pre-weighed vial. The wet weight was obtained with use of a stainless steel hook. The tissue samples were dried to a constant weight in a drying oven (90°C).

Analytical methods. Urine osmolality was measured by a Vapor Pressure Osmometer (Wescor Co., Ogden, Utah). Potassium and sodium in urine and plasma were measured by flame photometry (Model 143, Instrumentation Laboratories, Lexington, Massachusetts). To measure PAH clearance and extraction ratio, radioactivity in aliquots of urine, arterial and venous plasma were counted in a liquid scintillation counter (Searle Delta 300, Searle Analytic, Inc., Chicago, Illinois). Samples were corrected for quenching; clearance and extraction ratio of PAH were calculated in a standard fashion. Measurement of urine and plasma inulin concentration was performed with an autoanalyzer (Technicon Instruments Corporation, Tarrytown, New York), and the inulin clearance was calculated. ^{125}I-albumin in plasma and inner medullary tissue samples were measured with a gamma counter (Biogamma II, Beckman Instruments, Inc., Irvine, California). Inner medullary plasma flow rate in ml/min/100 g inner medullary weight was calculated using the formula:

$$\text{IMPF} = (\text{cpm/ml/100 g IM})/(\text{cpm/ml plasma}) \times (60 \text{ sec/min})/(\text{perfusion time(s)})$$

One-way analysis of variance and the Student-Newman-Keul multiple comparison test were used to analyze the data. The data are expressed as the mean ± 1 SEM.

Results

Table 1 summarizes body weight, plasma K concentration, urine flow rate, K excretion rate, GFR, CPAH, and the extraction ratio of PAH measured in control and K-depleted rats in the absence (groups 1 to 3) and in the presence of indomethacin (groups 4 to 5). There were no statistically significant differences in body weight among the five groups studied, although long-term K-depleted rats (24 days) tended to weigh less due to failure to grow and some weight loss during

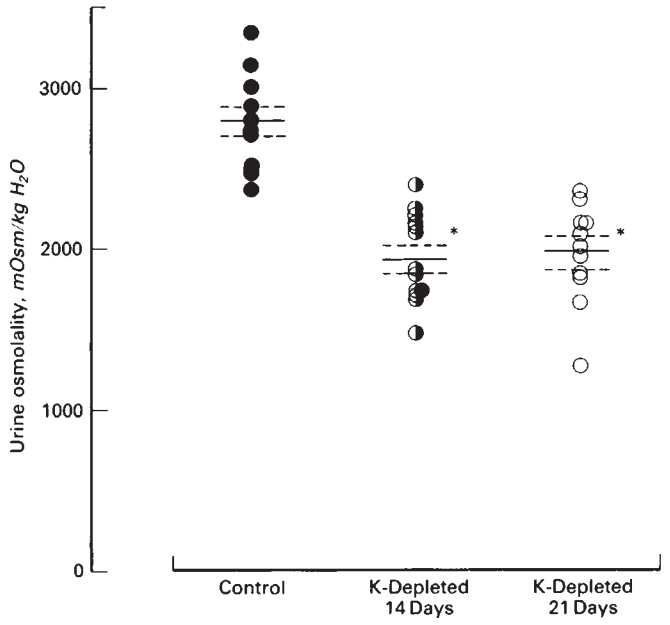

Fig. 1. *Development of a renal concentrating defect in potassium-depleted rats.* Maximum urine osmolality measured after 15 hr of dehydration in rats K-depleted for 14 days (◑, $N = 13$), and 21 days (○, $N = 12$), compared to control rats (●, $N = 10$). The *asterisk* indicates $P < 0.01$ compared to control. *Parentheses* indicate the number of animals.

the course of the dietary treatment. As expected, plasma K concentration was significantly lower in K-depleted rats, and they excreted 96% less potassium than control rats. For the purpose of comparison, GFR has been calculated per gram of kidney weight and per 100 g of body weight. Although GFR tended to be lower in K-depleted rats when expressed per 100 g of body weight, there were no significant differences. However, when expressed per gram of kidney weight, a substantial decrease in GFR in the K-depleted animals becomes evident. GFR is reduced by nearly 50% in all K-depleted groups. The clearance of PAH was strikingly decreased in rats K-depleted for 16 and 24 days to values less than 50% of control. The extraction ratio of PAH was not altered by 16 or 24 days of K-deficiency, but a statistically significant reduction was measured in long-term K-depleted animals treated with indomethacin (group 5). The data indicate that renal plasma flow is significantly reduced in the K-depleted animals (groups 2, 3, and 5). Estimated renal plasma flow in control and K-depleted rats are: control, 3.46; K-depleted 16 days, 1.52; and K-depleted 24 days; 1.66 ml/min per gram kidney weight. The filtration fractions calculated from the mean values of C_{PAH}, extraction ratio of PAH, and GFR are: control, 33.5%; K-depleted 16 days, 34.6%; and K-depleted 24 days, 31.1%.

As illustrated in Figure 1, a significant urinary concentrating defect developed in K-depleted rats within 14 days of dietary K deprivation. Maximum urine concentration measured after 15 hr of water deprivation was reduced in 14-day K-depleted rats (1937 ± 76.0 $mOsm/kg_{H_2O}$) compared to control rats (2801 ± 92.5 $mOsm/kg_{H_2O}$) but was not further accentuated by the continuation of K-deficiency for 21 days (1971 ± 83.7 $mOsm/kg_{H_2O}$).

Inner medullary plasma flow. Inner medullary plasma flow, measured by the accumulation of ^{125}I-albumin, was significantly reduced in rats K-depleted for 24 days (group 1, 35.1 ± 1.93) versus group 3, 13.8 ± 1.84 ml/min/100 g of IM weight, ($P < 0.01$, Fig. 2). However, IMPF was similar to control values in rats K-depleted for a duration of only 16 days (group 2, 32.8 ± 1.52).

To determine if the reduction of IMPF in group 3 animals was due to an increase in vasoconstrictor products of the renal cyclo-oxygenase system, IMPF was measured in control and long-term K-depleted rats given indomethacin acutely (groups 4 and 5, respectively). Treatment of control rats with 5 mg/kg of indomethacin produced no changes in GFR or renal plasma flow. There was a slight decrease in the extraction ratio of PAH, however, the change was not significant. There was also no change in IMPF in control rats treated with indomethacin (group 1, 35.1 ± 1.93 vs. group 4, 34.6 ± 3.45 ml/min/100 g IM weight, Fig. 2). There was no change in GFR or the clearance of PAH in long-term K-depleted rats given indomethacin. However, the data indicate there was a 32% increase in renal plasma flow in K-depleted rats given indomethacin (group 3, 1.67 vs. group 5, 2.20) since there was a significant decrease in the extraction ratio of PAH in group 5 rats (group 3, 0.76 ± 0.015 vs. group 5, 0.61 ± 0.014, $P < 0.01$). Despite the apparent increase in renal plasma flow, IMPF increased slightly, but the increase did not achieve statistical significance when compared to untreated long-term K-depleted rats (group 3, 13.8 ± 1.84 vs. group 5, 18.1 ± 1.63, ml/min/100 g IM). IMPF measured in group 5 K-depleted rats remained significantly lower than either control groups 1 or 4 (group 5 vs. group 1, 35.1 ± 1.93 and group 4, 34.6 ± 3.44 ml/min/100 g IM, $P < 0.01$, Fig. 2).

As reported by other investigators [18, 19], K-depleted rats fail to increase body weight at the same rate as pair-fed control rats, however, renal growth continues. These two factors taken together lead to a significant increase in the ratio of total renal mass in grams (kidney weight) to body weight (100 g body weight). After 2 weeks on the K-deficient diet, the ratio of kidney weight to body weight is increased by 35% compared to control rats and increases further (65%) in animals maintained on the diet for 24 days compared to control rats (Table 2). In the K-depleted kidney, however, the ratio of inner medullary mass to the mass of the kidney remains within normal limits (Table 2). The increase in renal mass is not due to fluid accumulation but rather to a true increase in tissue dry weight as evidenced by the significant reduction in tissue water of the inner medulla measured in K-depleted rats (Table 2).

Discussion

The mechanisms underlying the renal concentrating defect associated with K-depletion are still largely unclear. One factor complicating the elucidation of this problem is that dietary K-depletion produces a continuum of time-dependent changes in systemic and renal function [13, 14, 20]. With respect to changes in water metabolism, it has been shown that polydypsia develops on approximately day 7 of K-depletion in the rat while renal concentrating ability remains normal until about day 14 [14].

Medullary sodium chloride accumulation depends on ascending limb sodium chloride reabsorption. This process is limited

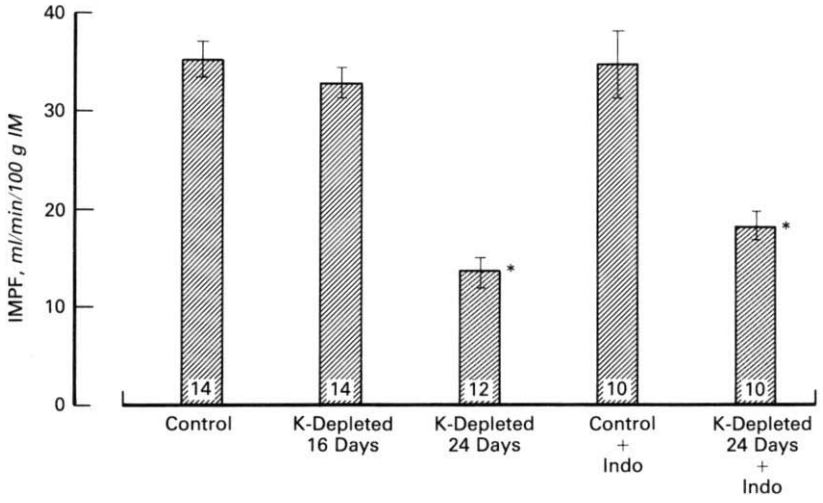

Fig. 2. *Changes in inner medullary plasma flow due to the duration of potassium depletion and treatment with indomethacin. Numbers* inside the *bar* equal the number of measurements. The *asterisk* indicates $P <$ 0.01 compared to groups 1, 2, and 4.

by the amount of sodium chloride delivered to the transporting segment. It is clear that GFR fixes the upper limit of total solute delivery to the loop. Decreases in GFR which are not offset by equivalent reductions in proximal sodium chloride reabsorption will decrease solute delivery to loops of Henle. To the extent that sodium chloride reabsorption by the ascending limb is reduced below the rate of solute removal via medullary blood flow, reductions in medullary sodium chloride would be expected to occur. There is controversy regarding the effect of dietary K depletion on GFR. Some [20–22] but not all [12, 23–25] studies report that GFR is decreased in K-depleted humans and animals. In the present investigation, GFR and C_{PAH} have been calculated as ml/min/100 g body weight and as ml/min/g kidney weight. GFR and C_{PAH} when calculated per 100 g body weight are not significantly reduced in K-depleted rats. However, the same functions when calculated per gram of kidney weight are severely reduced to nearly 50% of control in the K-depleted rats. The reason for this difference relates to the disruption of the normal relation between renal growth and body weight gain in K-depletion. It has been previously reported that K-depletion causes renal hypertrophy. Two studies have shown that within 15 days of consuming a K-free diet there is a doubling of renal mass, but only a 5 to 10% difference in body weight compared to pair-fed control rats [12, 18]. Biochemical studies reveal a net increase in protein synthesis and evidence of hyperplasia as well as hypertrophy [19]. As reported in the present study and other studies [12, 18, 19], the increase in kidney size reflects a true increase in renal mass, since tissue water content is not increased in K-depleted kidneys. In addition, the increase in mass is accompanied by a potential increase in filtering renal tissue. GFR [26] and RBF [20] when calculated as ml/min/g kidney weight have been shown to be severely reduced in the enlarged kidneys of rats K-depleted for 14 days. In one study [26], GFR was returned to normal values per gram of kidney weight following acute restoration of total body K. RBF [20] was acutely increased to normal values per gram kidney weight in K-depleted rats treated with saralasin and indomethacin. Therefore, the hypertrophied kidneys of K-depleted rats appear to be capable of normal rates of glomerular filtration and renal blood flow. If renal mass increases without a proportionate increase in GFR, then solute delivery to loops of Henle will not be adequate for the increase in tissue growth. Although all areas of the kidney grow in K-depletion, there is striking proliferation of the outer medulla [27]. The reduction in GFR in kidneys undergoing hypertrophy in K-depleted animals may be a major contributing factor to diminished medullary solute content [3] in this electrolyte disorder.

Table 2. The effect of 2 and 3 weeks of K-depletion on inner medullary water content and ratios of kidney weight to body weight and inner medullary weight to kidney weight[a]

Condition of rats	Inner medullary H_2O content %	KW/BW[b]	IM/KW[c]
Control	87.3 ±0.37 (16)	0.82 ±0.028 (10)	0.0357 0.00134 (14)
K-depleted 16 days	84.3[d] ±0.35 (18)	1.11[e] ±0.096 (13)	0.0366 0.00264 (16)
K-depleted 24 days	83.6[d] ±0.36 (14)	1.36[d] ±0.101 (7)	0.0419 0.0042 (12) NS

[a] The number of measurements is in parentheses.
[b] The values represent the ratio of the weight of two kidneys in grams to 100 g of body weight.
[c] The values represent the ratio of the weight of each inner medulla to the weight of the same kidney in grams.
[d] $P < 0.01$ compared to control rats.
[e] $P < 0.05$ compared to control rats.

Since there is evidence that alterations in IMPF may have important effects on renal concentrating ability [9–11]; the finding that IMPF was reduced in rats K-depleted for approximately 3 weeks was considered to be a factor contributing to the presence of the renal concentrating defect [12]. In that study, the reduction in IMPF was thought to reflect decreased plasma delivery to juxtamedullary nephrons which may have reduced solute delivery to the loop of Henle. If the reduction in IMPF in long-term K-depleted rats is associated with the initiating factor responsible for the development of the concentrating defect, then this change should be present in rats K-depleted for only 2 weeks at which time the defect first appears. The results of the present study show that after 2 weeks of K-depletion the

concentrating defect is well established, however IMPF measured by the ^{125}I-albumin accumulation method is not significantly different compared to IMPF measured in control rats. In addition, I have confirmed the previous finding [12] that IMPF is significantly reduced in rats K-depleted for more than 21 days. The results derived from the present study support the conclusion that the development of the renal concentrating defect due to K-depletion does not depend on changes in IMPF. More specifically, the reduction in medullary solute content [3] and the presence of the renal concentrating defect after 2 weeks of K-depletion cannot be attributed to a reduction in IMPF.

Although the reduction in IMPF measured in long-term K-depleted rats is not necessary for the development of the renal concentrating defect, the mechanism underlying this change is of some interest. There is evidence that inner medullary synthesis of the vasoconstrictor, thromboxane A_2 (assessed by measurement of thromboxane B_2, its stable metabolite) is increased in tissue derived from rats K-depleted for 3 weeks [15] while the synthesis of PGE_2 (a vasodilator in the rat) is not increased [15, 20]. Since products of the cyclo-oxygenase enzyme system may exert effects on IMPF [9]; IMPF was measured in long-term K-depleted rats treated with indomethacin. The effect of indomethacin in K-depleted rats should be the reduction of elevated levels of the vasoconstrictor thromboxane A_2. Additional evidence supporting this view comes from the study by Linas and Dickman [20] in which equivalent increases in renal blood flow in K-depleted kidneys were obtained with indomethacin or imidazole (a specific inhibitor of thromboxane synthesis). If other products of the cyclo-oxygenase enzyme system had major effects on renal hemodynamics in K-depletion, one would not expect the effects of inhibiting the entire pathway to be similar to the effects of inhibiting only a small portion of the same pathway. With regard to the effect of inhibition of the cyclo-oxygenase enzyme system on renal hemodynamics in the present study there was a similar increase in RPF (32%) estimated from the extraction ratio of PAH and the clearance of PAH in long-term K-depleted rats. The decrease in the extraction ratio of PAH in the presence of indomethacin observed in this group is consistent with an earlier report [28] that indomethacin is a substrate for the organic acid transport system. The extraction ratio of PAH decreased slightly in control rats treated with indomethacin, but the change was not statistically significant. That indomethacin inhibits PAH transport more in long-term K-depleted rats may be related to the relative decrease in cortical mass [27] which occurs after 3 to 4 weeks of dietary K-depletion.

In contrast to the effect of indomethacin on RPF in long-term K-depleted rats, the results of the present study show that IMPF is not significantly increased after treatment with this cyclo-oxygenase inhibitor. This finding appears to conflict with a recent report by Takamitsu and Kunau [29]. In that study, however, treatment with indomethacin was carried out for 3 days prior to measurement of IMPF. In addition, although IMPF was slightly increased following treatment with indomethacin, values measured in K-depleted rats were still greatly reduced compared to the control rats in that study. Therefore, it appears that vasoconstrictor products of the cyclo-oxygenase enzyme system do not play a major role in the reduction in IMPF measured in long-term K-depleted rats. In addition, the results of the present study show that the decrease in IMPF in long-term K-depleted rats is not due to a disproportionate increase in inner medullary size. In conclusion, the renal concentrating defect which develops after 2 weeks of dietary K-deprivation occurs independently of changes in IMPF and therefore must be due to other factors. Recent results from our laboratories [26] using a modified loop microperfusion technique in vivo, suggest that thick ascending limb transport is significantly impaired after 2 weeks of K-depletion; the defect in loop transport correlates with the decrease in plasma K concentration in K-depleted rats and is reversed when K is administered acutely. Impaired sodium chloride transport by the thick ascending limb coupled with reduced solute delivery to loops of Henle (estimated from the decrease in GFR) would be expected to contribute in a major way to the development of the concentrating defect in K-depletion.

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